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Microbial Responses to Biochar Soil Amendment and Influential Factors: A Three-Level Meta-Analysis

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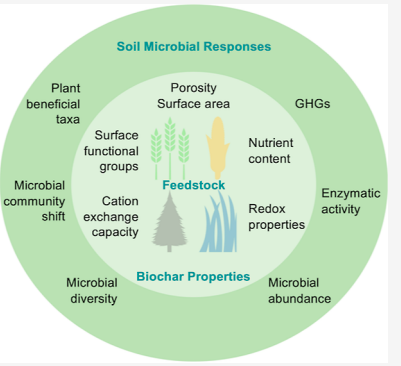
ABSTRACT: Biochar is a multifunctional soil conditioner capable of enhancing soil health and crop production while reducing greenhouse gas emissions. Understanding how soil microbes respond to biochar amendment is a vital step toward precision biochar application. Here, we quantitatively synthesized 3899 observations of 24 microbial responses from 61 primary studies worldwide. Biochar significantly boosts microbial abundance [microbial biomass carbon (MBC) > colony-forming unit (CFU)] and C- and N-cycling functions (dehydrogenase > cellulase > urease > invertase > *nirS*) and increases the potential nitrification rate by 40.8% while reducing cumulative N_2O by 12.7%. Biochar derived at lower pyrolysis temperatures can better improve dehydrogenase and acid phosphatase and thus nutrient retention, but it also leads to more cumulative CO_2. Biochar derived from lignocellulose or agricultural biomass can better inhibit N_2O through modulating denitrification genes *nirS* and *nosZ*; repeated biochar amendment may be needed as inhibition is stronger in shorter durations. This study contributes to our understanding of microbial responses to soil biochar amendment and highlights the promise of purpose-driven biochar production and application in sustainable agriculture such that biochar preparation can be tuned to elicit the desired soil microbial responses, and an amendment plan can be optimized to invoke multiple benefits. We also discussed current knowledge gaps and future research needs.



KEYWORDS: *biochar, sustainable agriculture, feedstock, pyrolysis temperature, soil microbiome, nutrient cycling, greenhouse gas, meta-analysis, mixed-effects model*

1. INTRODUCTION

Soil is a vital ecosystem that sustains food security and other development goals.[1] During the past decade, biochar has received increasing attention due to its promise as a low-cost, multifunctional soil conditioner (Figure S1).[2] Numerous studies have reported that biochar soil amendment is capable of preserving or improving soil quality, promoting crop production, decreasing nutrient leaching, and reducing greenhouse gas (GHG) emissions from agricultural soils.[3−5] Yet, the mechanisms underlying biochar's beneficial effects on the soil−plant continuum are not fully understood, which hinders the realization of the full potential of biochar in sustainable soil management.

Soil contains a vast diversity of microorganisms that together mediate soil functions and directly contribute to plant fitness in a changing environment.[6,7] Biochar amendment can alter the indigenous soil microbiome, which could, in turn, drive shifts in soil functionality. Indeed, studies around the world have identified significant alterations of soil microbial biomass, diversity, community composition, and enzyme activity following biochar addition (e.g., refs 8−11). Some, however, found only minor changes in these perspectives (e.g., refs 12−14).

The large variations and sometimes contrasting outcomes across the studies could be attributed to heterogeneity in soil characteristics, biochar properties, or amendment protocols. First, soil is a complex matrix varying in abiotic properties [e.g., pH and cation exchange capacity (CEC)] and biotic components (e.g., indigenous soil microbiome). Second, biochar can be derived by the thermochemical conversion of a wide range of organic materials (i.e., feedstocks) under oxygen-limiting conditions. Among various biochar preparation methods, pyrolysis is more widely adopted than other methods, such as hydrothermal carbonization and gasification, due to its simple operation at a range of scales. Biochar physicochemical properties, such as porosity, aromaticity, and surface functional groups, vary considerably depending on the feedstock and conversion process parameters, particularly temperature.[15−17] Third, soil amendment protocols can differ

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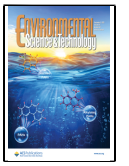

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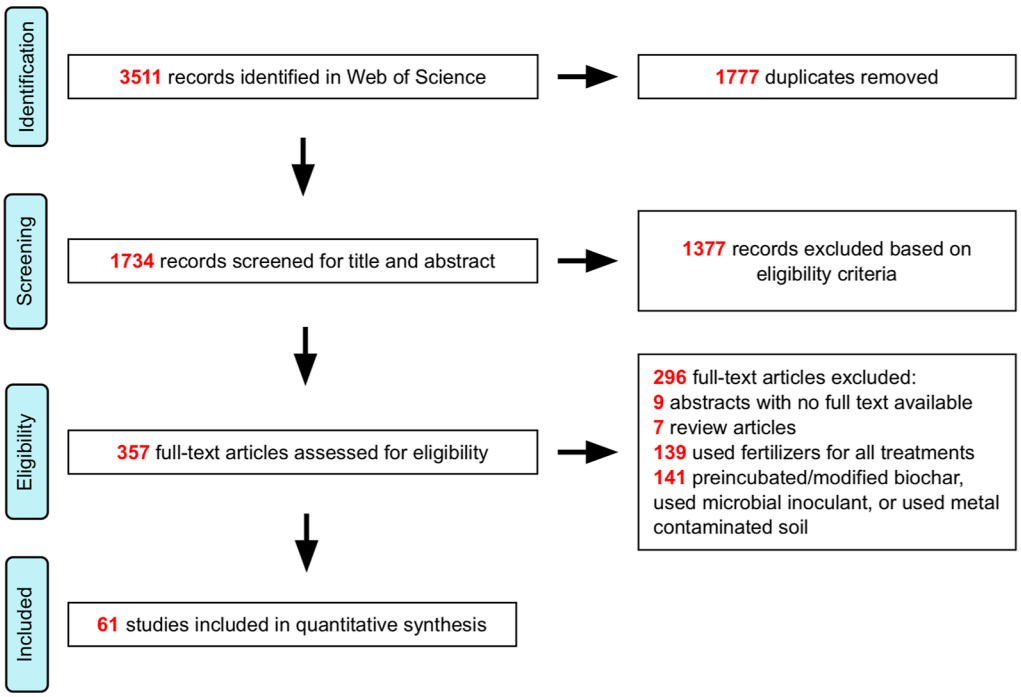

Figure 1. PRISMA flow diagram summarizing the different phases of the systematic literature review, study screening and exclusion details, and the number of studies retained for quantitative analysis.

substantially in frequency, rate, and duration, all influencing amendment outcomes.[18]

A systematic review of the current literature would allow us to synthesize knowledge of soil microbiome responses to biochar amendment, unveil key factors that influence biochar effects on the soil microbiome, and identify significant knowledge gaps in the field. Specifically, meta-analysis quantitatively analyzes the results from multiple primary studies, allowing more robust conclusions to be drawn and new insights to be gained.[19] Meta-analysis has been employed to investigate biochar effects on soil quality,[20] crop production,[4] GHG emission,[21,22] and more recently microbial variables as well.[11,23−25] However, influential factors for microbial responses have not been quantitatively analyzed.

Here, we utilized a three-level meta-analysis framework to quantitatively synthesize the most recent global findings of biochar impacts on the soil microbiome, examined the influence of biochar characteristics, soil properties, and amendment protocols on soil microbial responses at the molecular, population, and community level, and ranked the potential drivers of microbial responses. This framework has a hierarchical structure of an extended mixed-effects model, allowing dependent observations to be retained and heterogeneity at different levels to be quantitatively assessed.[26,27] Our results provide novel insights into biochar's benefits to the soil−plant continuum and underscore the promise of purpose-driven biochar amendment for sustainable agriculture.

2. MATERIALS AND METHODS

2.1. Systematic Literature Review. A systematic literature search was performed using the keyword "biochar" paired with soil microbial response terms (listed in Table S1) in the Web of Science to identify relevant peer-reviewed articles. Publications were limited by date (between January 2018 and April 2020) and manuscript type (original research). This resulted in an initial collection of 3511 publications from which 1777 duplicates were removed. Publications were further screened according to the following inclusion criteria: (1) experimental design allowed pairwise comparison between biochar treatment and no-biochar control, (2) if fertilizer was applied, both biochar-only and no-fertilizer controls were available to isolate the effects of biochar, (3) soil used was not contaminated with metal or mine waste, (4) biochar was produced by pyrolyzing organic materials under dry conditions and was not preincubated, (5) microbial inoculants were absent, and (6) the study reported sample sizes, means, and standard errors or the information was given by the author through personal communication. This resulted in 61 studies (see Supporting Information references), including 14 field studies and 3899 pairwise treatment−control comparisons. Figure 1 shows a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) diagram summarizing the screening procedure.[28] More details are in the Supporting Information.

2.2. Data Extraction. We examined the 61 studies carefully to extract data, including experimental designs, biochar production conditions and characteristics, soil properties, soil biogeochemistry, soil (including rhizosphere) microbial responses, and plant responses when available. Data were collected directly from original tables where possible; data only presented in figures were extracted using WebPlotDigitizer (v4.2); authors were contacted for data not explicitly reported in the publications. A variety of response variables were obtained (Table S2), and those with ≥20 observations were included in the meta-analysis. Similar to others, we initially assigned predictor variables (moderators) to categorical groups to facilitate cross-study comparisons, considering the distribution of categorical levels in the entire database (Table S3). Biochar feedstocks were grouped into the following categories: (1) manure (poultry, pig, or cattle manure), (2) sludge (water treatment plant sewage sludge), (3) wood (hardwoods such as pine, oak, beech, fir, bamboo, and wood mixtures), (4)

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agricultural biomass (residues from rice, wheat, corn, sugar cane, and legumes), and (5) lignocellulose (nut shells, fruit peels, weeds, and tree leaves). If soil texture was not reported in a study, the harmonized world soil database[21,29,30] was used to extract soil texture information according to the reported coordinates. When soil organic matter (SOM) rather than soil organic carbon (SOC) was reported, SOC was estimated as 58% of SOM.[31] Biochar application rates reported as metric tons/hectare were converted to mass percentages using the reported soil bulk density.

2.3. Three-Level Meta-analytical Model. We followed the general methodology in Koricheva et al. (2013),[32] from data compiling to statistical modeling, to conduct a meta-analysis on the extracted data. The effect sizes of the biochar treatments on soil microbial responses, as well as soil or plant responses, were measured using the log response ratio (LRR)[33]

$$\mathrm{LRR}=\ln\left(\frac{\bar{X}_b}{\bar{X}_c}\right) \tag{1}$$

where $\bar{X}_b$ is the mean of a response variable under biochar treatment and $\bar{X}_c$ is the mean of the response variable under the control.

The estimate of the study variance ($\hat{\sigma}^2$) was calculated using the reported variance of the mean of a response variable

$$\hat{\sigma}^2 = \frac{\mathrm{SD}_b^2}{n_b \times \bar{X}_b^{\,2}} + \frac{\mathrm{SD}_c^2}{n_c \times \bar{X}_c^{\,2}} \tag{2}$$

where SD^2 indicates the reported standard deviation of the mean of the response variable and n is the sample size or reported number of replicates. We used a three-level random-effects model for effect sizes, assuming random effects at different levels and independent sampling error[34]

$$y_{ij} = \beta_0 + u_{(2)ij} + u_{(3)j} + e_{ij} \tag{3}$$

where y_{ij} is the ith effect size in the jth study; β_0 is the average population effect; $\mathrm{Var}(e_{ij}) = v_{ij}$ with e_{ij} is the sampling error of the ith effect size in the jth study (level 1); $\mathrm{Var}(u_{(2)ij}) = \tau^2_{(2)}$ is the within-study heterogeneity (level 2); and $\mathrm{Var}(u_{(3)j}) = \tau^2_{(3)}$ is the between-study heterogeneity (level 3). We assumed that the marginal errors and random effects were normally distributed with a mean of 0 and were independent. We used the statistic I^2 to estimate the proportion of variation in the effect sizes explained by level-2 or level-3 variance, with total heterogeneity being the sum of both,[35] and Cochran's Q to test the significance of heterogeneity (also see the Supporting Information).[36] Model parameters were estimated using restricted maximum likelihood (REML).[37] Sources of high I^2 were explored through extending eq 3 to a three-level mixed-effects model with individual moderators as a covariate[38]

$$y_{ij} = \beta_0 + \beta_1 x_{ij} + u_{(2)ij} + u_{(3)j} + e_{ij} \tag{4}$$

where β_1 is a moderator regression coefficient; x is a moderator covariate at either level 2 (x_{ij}) or level 3 (x_j; same for all effect sizes in the jth study); conditional mean $E(y_{ij}|x_{ij}) = \beta_0 + \beta_1 x_{ij}$; conditional variance $\mathrm{Var}(y_{ij}|x_{ij}) = \tau^2_{(2)} + \tau^2_{(3)} + v_{ij}$; conditional covariances $\mathrm{Cov}(y_{ij}, y_{kj}|x_{ij}) = \tau^2_{(3)}$ (i.e., the same covariance is shared by effect sizes in the jth study); $\mathrm{Cov}(y_{ij}, y_{mn}|x_{ij}) = 0$ (i.e., independent effect sizes are assumed for different studies); $\tau^2_{(2)}$ and $\tau^2_{(3)}$ are the level-2 and level-3 residual heterogeneity, respectively, after controlling for the covariate. Nine moderators were considered: biochar characteristics (feedstock, pyrolysis temperature), soil properties (pH, C/N, CEC, bulk or rhizosphere), and treatment protocols (biochar application rate, fertilization, experiment duration, field or laboratory). After analyzing the individual moderators, we extended the three-level mixed-effects model to contain multiple moderators. Here, soil pH, soil C/N, experiment duration, and biochar application rate were treated as continuous predictors, while others were categorical. Soil type, fertilization, and experiment type were excluded due to the low number of observations and/or overlapping with other moderators. Meta-regression models considering only main effects (i.e., without interactions) were fitted based on log-likelihoods. Multicollinearity of candidate predictors was evaluated using the variance inflation factor (VIF). Non-correlated predictors were included in automated model selection for each response variable. The most parsimonious model was selected using the corrected Akaike information criterion (AICc), and the model-averaged moderator importance was calculated. Maximum likelihood estimation instead of REML was used to allow the use of AIC to compare for models with different fixed effects.[39] The percentage of heterogeneity explained by the inclusion of one or more moderators was estimated using a pseudo-R^2 statistics[40]

$$\text{pseudo-}R^2 = (\Sigma\sigma^2_{\mathrm{Model1}} - \Sigma\sigma^2_{\mathrm{Model2}})/\Sigma\sigma^2_{\mathrm{Model1}} \tag{5}$$

where $\Sigma\sigma^2_{\mathrm{Model1}}$ is the sum of residual variances of the random intercept components in the overall model (no moderator) and $\Sigma\sigma^2_{\mathrm{Model2}}$ is the sum of residual variances of the random intercept components in a model with one or more moderators for a given response variable. If the pseudo-R^2 calculation generated negative values, then they were truncated to zero. Additional modeling details including multimodel inference are given in the Supporting Information.

2.4. Publication Bias and Missing Data. Techniques for handling missing data in conventional meta-analysis have not been comprehensively evaluated for multilevel meta-analysis.[38] Here, we utilized multiple techniques, before and after model selection, to better assess and handle missing data due to publication bias (details in the Supporting Information).

2.5. Computational Implementation. All analyses were performed in R (v3.6.1) with package "metafor" for meta-analysis, "corrplot" and "performance" for collinearity examination, "glmulti" for automated stepwise model selection, and "ggplot2" for data visualization.[40−44]

3. RESULTS AND DISCUSSION

3.1. Global Estimate of Microbial Responses to Biochar Soil Amendment. We obtained 24 response variables with ≥20 observations from 61 primary studies and 3899 pairwise treatment−control comparisons (Table S4). These included microbial abundance [bacterial colony-forming unit (CFU), bacterial phospholipid fatty acid (PLFA), fungal PLFA, microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN)], diversity (ACE, Chao1, Shannon, and Simpson), gene abundance (archaeal *amoA*, bacterial *amoA*, *narG*, *nirS*, and *nosZ*), enzyme activity (acid phosphatase, alkaline phosphatase, β-glucosidase, cellulase, dehydrogenase, invertase, and urease), and process (cumulative CO_2, cumulative N_2O, potential nitrification rate). The genes, enzymes, and processes are involved in C, N, and P cycling. Except for CFU and ACE, our three-level random-

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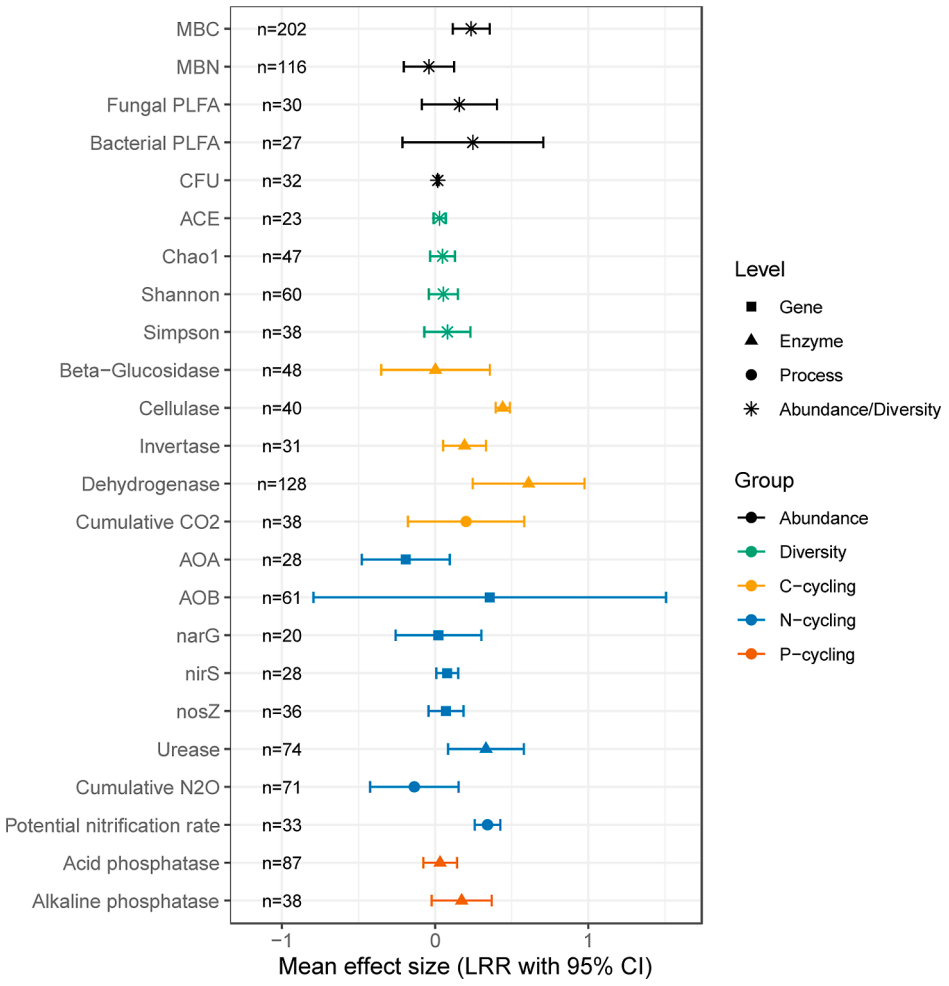

Figure 2. Mean effects of biochar on soil microbial responses (weighted LRR ± 95% confidence interval). Effect sizes where 95% confidence intervals do not overlap with zero are significant. AOA, ammonium-oxidizing archaea; AOB, ammonium-oxidizing bacteria; CFU, colony-forming unit; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; PLFA, phospholipid fatty acid. AOB and AOA were commonly measured with quantitative PCR targeting the ammonia monooxygenase gene *amoA*.

effects model could explain 77.7−99.9% of the total heterogeneity in these variables (in the Supporting Information).

Biochar soil amendment enhanced 21 of the 24 response variables (Figure 2; Table S5). Such increases were significant for two abundance variables CFU (+1.74%, n = 32) and MBC (+26.5%, n = 202), *nirS* gene encoding nitrite reductase that catalyzes the conversion of nitrite to nitric oxide in denitrification (+8.2%, n = 28), cellulase that decomposes cellulose and related polysaccharides (+55.6%, n = 40),[45] dehydrogenase that catalyzes soil organic matter oxidation (+84.1%, n = 128),[46,47] invertase that attacks β-D-fructofuranoside in oligosaccharides (+21.2%, n = 31),[48] urease that hydrolyzes urea to ammonia and CO_2 (+39.4%, n = 74), and potential nitrification rate (+40.8%, n = 33). Biochar insignificantly decreased 3 variables: MBN (−4.0%, n = 116), ammonium-oxidizing archaea (AOA) (−17.4%, n = 28), and cumulative N_2O (−12.7%, n = 71). Ammonium-oxidizing bacteria (AOB) had the largest response variance (−54.8 to +351.8%, n = 61, 5 studies), which was not due to within- or between-study variation alone (49.4 and 50.6% of total heterogeneity, respectively). Overall, these global estimates highlight that biochar can modulate the soil microbiome, resulting in a wide range of alterations at the molecular, population, and community level.

3.2. Biochar Effects on Soil Microbial Functions Related to C,N,P Cycling. Biochar significantly stimulated the activity of three C-cycling enzymes: cellulase, dehydrogenase, and invertase (Figure 2; Table S5). Cellulase is responsible for the turnover of plant biomass in the biosphere, and there are three major types produced by certain bacteria and fungi: 1,4-β-cellobiosidase (EC 3.2.1.91/176), *endo*-1,4-β-D-glucanase (EC 3.2.1.4), and β-glucosidase (EC 3.1.2.21).[45] Biochar enhanced the activity of cellulase in general but had no significant effect on the activity of β-glucosidase that completes the final step of cellulose hydrolysis. Biochar strongly enhanced intracellular dehydrogenase (EC 1.1.1.) activity. This is likely attributed to improved soil aeration, water availability, pH, and nutrient availability that are known to significantly influence microbial biomass and thus dehydrogenase activity.[16,46,47,49] Two earlier meta-analyses focused specifically on enzyme activities found that biochar had insignificant or negative effect on 1,4-β-cellobiosidase and β-glucosidase and positive effect on dehydrogenase.[9,11] It should be noted that since our analysis included all three cellulase types, the results may reflect a more comprehensive view. Fewer studies have investigated invertase (EC 3.2.1.26), which is widely distributed in microorganisms,

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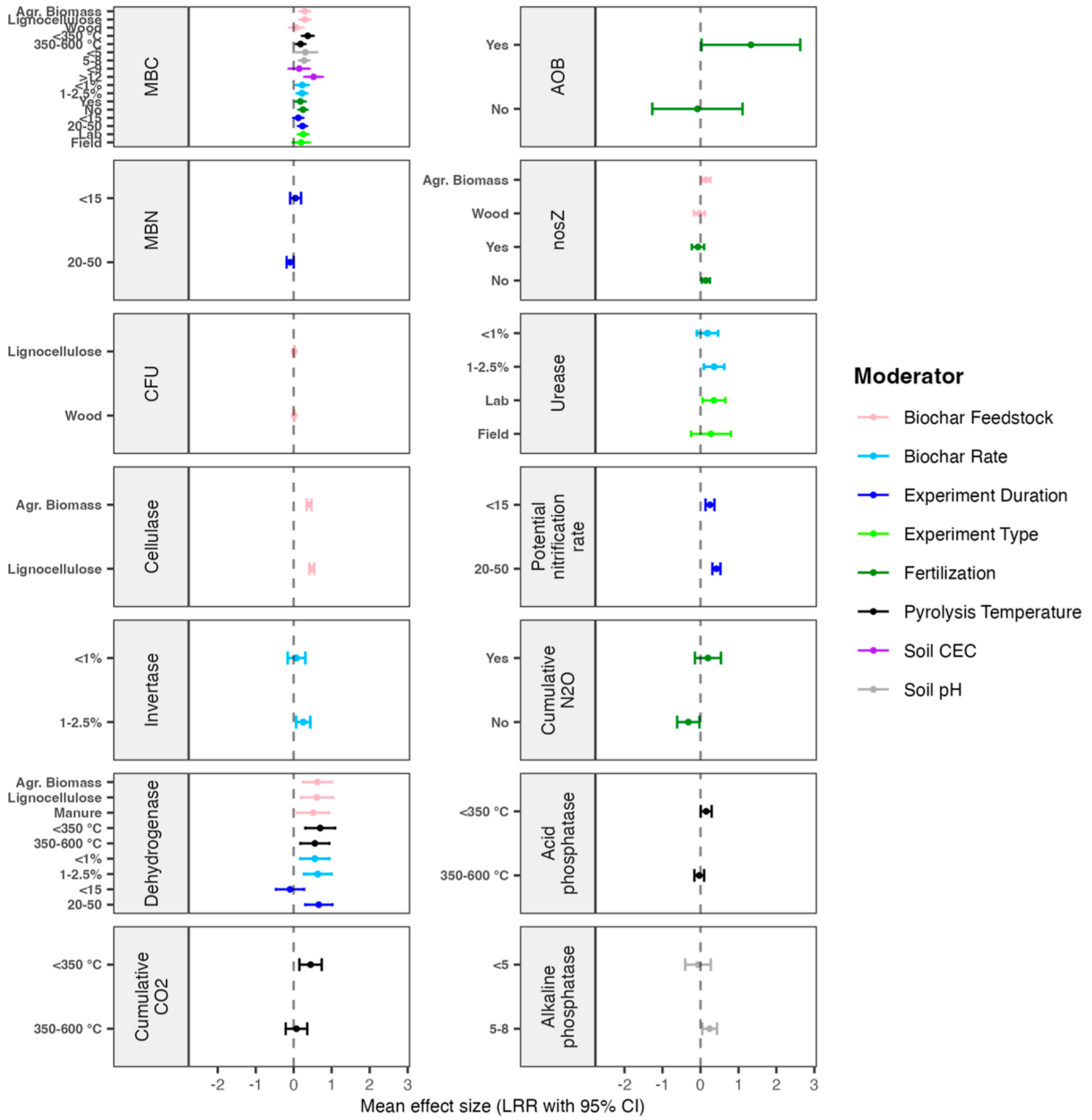

Figure 3. Mean effects of individual moderators on soil microbial responses (weighted LRR ± 95% confidence interval). Data presented here had >10 observations at each moderator level (data with <10 observation at certain moderator levels are provided in the Supporting Information and Figure S3). No significant effect was seen in β-glucosidase activity.

both intracellular and extracellular.[48] Individual studies have reported both increased and decreased invertase activity, depending on soil type, biochar dose, and experimental design.[50,51] Our results based on data from 7 studies suggested generally enhanced invertase activity. Overall, biochar-induced higher activity of C-cycling enzymes may result in faster C turnover, and indeed, our estimate showed a 22.4% increase in cumulative CO_2 due to biochar amendment, similar to another report stating 19% increase.[22] These results are further supported by a recent study showing biochar-induced bacterial mineralization of soil recalcitrant components.[52]

N cycle is driven by complex networks of metabolically versatile microorganisms.[53,54] Among N-cycling-related responses, biochar significantly stimulated the *nirS* gene, urease (EC 3.5.1.5) activity, and potential nitrification rate, while reducing AOA and cumulative N_2O although not significantly. These results are generally consistent with previous reports that biochar can increase urease activity, AOA abundance, nitrification process, and the abundance of denitrification gene *nirS* encoding nitrite reductase ($NO_2^- \rightarrow NO$) and *nosZ* encoding nitrous oxide reductase ($N_2O \rightarrow N_2$) but has no significant effect on AOB.[9,11,16,24] Among these biochar effects, the inhibition of soil N_2O emissions has received increasing research interest. Nitrous oxide is 310 times more potent than CO_2 and accumulates in the atmosphere at a rate of 2% per decade.[55] Two-thirds of global N_2O emissions originate from soil, particularly agricultural soils with excessive N fertilizers. Our analysis found that biochar could reduce cumulative N_2O by 12.7% (71 observations from 11 studies), although substantial within-study variation (80.8% of total heterogeneity) limited this effect's significance. This finding is generally consistent with other meta-analyses focused specif-

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ically on N_2O emissions, which reported an overall 16−50% N_2O reduction by biochar.[5,21,22] Taken together, our results showed a 40.8% increase in potential nitrification rate and a 12.7% reduction in cumulative N_2O, thus strong inhibition of denitrification, by biochar. This finding is in line with the observation that biochar increased *nirS* (8.2%, $p < 0.032$) and *nosZ* (7.4%, $p = 0.216$) genes. We noted that genes encoding diverse nitric oxide reductases (NOR) that directly produce N_2O[53] are rarely monitored in biochar soil amendment. Future research may quantify the effects of biochar on NOR genes and enzyme activity.

Biochar enhanced the activity of two P-cycling enzymes despite the lack of significance, and alkaline phosphatases showed greater enhancement than acid phosphatases (18.9 vs 3.3%). Phosphatases hydrolyze phosphomonoesters and to a lesser extent phosphodiesters, releasing phosphate.[47] Higher phosphatase activity could increase P availability, which is commonly seen in biochar-treated soils.[18] One meta-analysis reported similar results to ours, but another meta-analysis reported insignificant decrease in acid phosphatase activity and significant increase in alkaline phosphatase activity.[9,11] These discrepancies likely reflect soil pH differences across studies which largely influences phosphatase activity, given that the two phosphatases have different optimal pH ranges.[11,56,57]

3.3. Influential Factors of Microbial Responses. Overall, biochar characteristics, soil properties, and treatment protocols, all influenced the effects of biochar on the soil microbiome (Figures 3 and S2). The inclusion of a moderator in the mixed-effects model reduced total nonsampling variance for all the response variables except for CFU (Table S6), but this occasionally lowered the number of observations at a particular moderator level (see the Supporting Information for comparisons between treatments having <10 observations).

3.3.1. Factors Influencing Microbial Abundance and Diversity. Microbial biomass has been considered a soil health indicator, and its increase is desired for soil functioning.[1] Biochar's positive effects on MBC were significantly influenced by all moderators except for soil C/N and soil zone ($p < 0.003$ in omnibus test) (Figure 3). Biochar produced from wood had the least enhancing effects on MBC (agricultural biomass: +34.39%, $n = 67$, $p < 0.001$; lignocellulose: +33.81%, $n = 33$, $p < 0.001$; wood: +7.38%, $n = 99$, $p = 0.466$). Low pyrolysis temperatures resulted in >2 times higher MBC increase than that with medium pyrolysis temperatures (≤350 °C: +45.11%, $n = 24$; 350−600 °C: +20.10%, $n = 178$; both $p < 0.01$), likely due to a higher amount of labile C and available nutrients in biochar produced at lower temperatures.[11] Larger MBC increases occurred in acid soil (pH < 5: +35.72%, $n = 75$, $p = 0.061$; pH 5−8: +31.84%, $n = 107$, $p < 0.001$), in soil with high CEC (>12 cmol/kg: +68.83%, $n = 11$, $p < 0.001$; <9 cmol/kg: +15.56%, $n = 46$, $p = 0.303$), at low and medium biochar application rates (<1% rate: +25.40%, $n = 74$; 1−2.5% rate: +24.54%, $n = 126$; both $p < 0.01$), without fertilization (no fertilizer: +27.83%, $n = 162$; fertilizer: +19.34%, $n = 40$; both $p < 0.015$), after longer duration (<15 days: +13.06%, $n = 48$, $p = 0.070$; 20−50 days: +26.09%, $n = 150$, $p < 0.001$), and in the laboratory (laboratory: +28.41%, $n = 169$, $p < 0.001$; field: +21.74%, $n = 33$, $p = 0.100$). These results are generally consistent with other reports of 21.7% MBC increase and larger increase by biochar generated from agricultural biomass and lignocellulose than that from wood or under lower than higher pyrolysis temperature.[11,25] However, they reported smaller MBC increases in acidic than neutral soil, after medium than short duration, and from laboratory than field studies. These discrepancies are partially due to categorization differences between our analysis and others; they defined acidic soil as pH < 6.5 and short duration as up to 100 days.[11] Discrepancies may also come from different measurements used to collect MBC data by the primary studies.[25] Interestingly, biochar increased rhizosphere MBC nearly 3 times more than that by bulk soil (Figure S2). Although the small sample size limits the generality of this finding (2 rhizosphere observations vs 200 bulk soil observations), it highlights the potentially profound biochar effects on the rhizosphere and warrants future research in this direction. For MBN, experiment duration was the only significant moderator ($p = 0.039$) (Figure 3). Shorter duration increased MBN (+5.32%, $n = 32$), while longer duration decreased MBN (−8.76%, $n = 80$), but neither was significant. These results, consistent with others,[11] suggest limited effects of biochar on MBN. For bacterial and fungal PLFA, feedstock was the only significant moderator ($p < 0.001$) (Figure S2). Biosolids biochar increased bacterial and fungal PLFA remarkably more than lignocellulose or wood biochar (Figure S2).

Below-ground biodiversity is a key factor for maintaining the functioning of soil ecosystems, and reduced microbial diversity could result in the decline of multiple soil functions including nutrient cycling and retention.[58] However, diversity indicators are not widely adopted for soil health measurements due to limited functional knowledge and lack of effective methods.[1] Here, observations at a particular moderator level were often less than 10 and occasionally all from one study (Figure S2; Table S6). Nonetheless, we found substantially higher diversity increases in the following comparisons: biochar made from biosolids versus other feedstocks, rhizosphere versus bulk soil, with fertilizer versus without fertilizer, and in the field versus in the laboratory. We found no significant difference in diversity change among biochar application rates and marginally larger diversity increases from shorter versus longer duration. Others reported similar observations. For example, one laboratory study found that biochar increased both prokaryotic and fungal diversity in the wheat rhizosphere, with greater diversity increases from medium (1−2%) than that from high (4%) application rate.[10] One meta-analysis focused specifically on microbial diversity reported greater Shannon index increases by biochar made from manure and sludge versus other feedstocks.[25] The same study also found larger bacterial diversity increases from field versus laboratory but larger fungal diversity increases from laboratory versus field.

3.3.2. Factors Influencing C,N,P-Cycling Functions and Processes. For C-cycling enzymes and cumulative CO_2, biochar feedstock, pyrolysis temperature, soil CEC, fertilizer addition, biochar application rate, and experiment duration were significant moderators ($p < 0.035$ in omnibus test) (Table S6). Significant factors for cellulase activity included biochar feedstock and the experiment duration. Lignocellulose biochar promoted cellulase activity more than agricultural biomass biochar (agricultural biomass: +50.11%, $n = 20$; lignocellulose: +61.22%, $n = 20$; both $p < 0.001$); longer experiments resulted in larger increases (<15 days: +42.14%, $n = 8$; 20−50 days: +59.14%, $n = 32$; both $p < 0.001$) (Figures 3 and S2). Significant moderators for invertase activity included biochar feedstock, pyrolysis temperature, fertilizer addition, and the biochar application rate. In general, larger invertase activity increases occurred by biochar made from agricultural biomass and at medium pyrolysis temperature (agricultural

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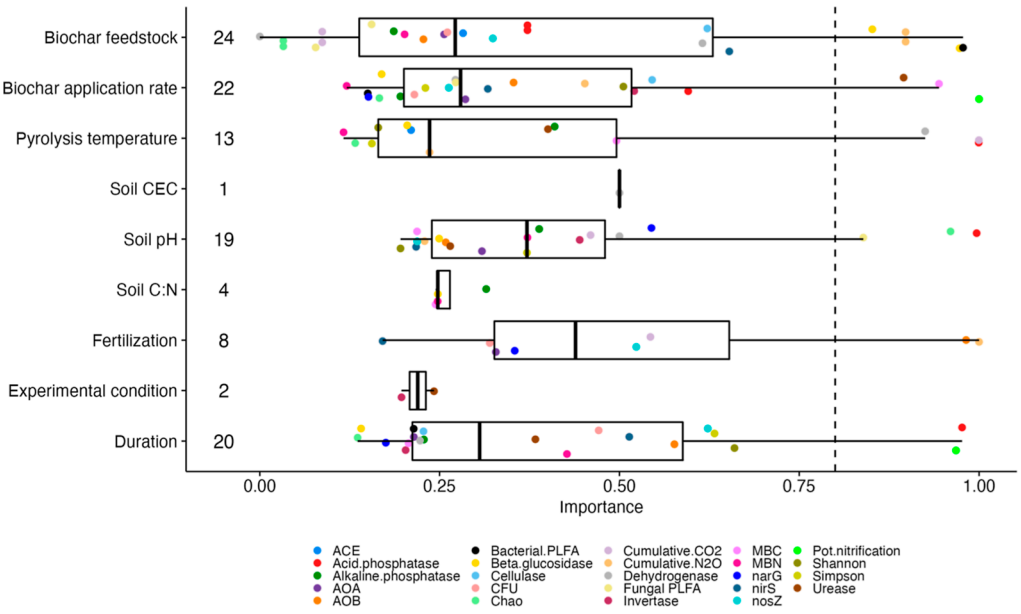

Figure 4. Contributors to microbial responses to biochar soil amendment. Model-averaged importance of the predictors for each soil microbial response was calculated as the sum of Akaike weights derived from the model selection using AICc (Akaike information criterion corrected for all samples). Box-and-whisker plots show minimum and maximum (whisker bottom and top), first and third quartile (box bottom and top), and median (line inside box) of model-averaged importance of different predictors, while colored dots show the importance of particular predictors to various microbial responses (also see Table S7). The number next to a predictor indicates the number of models containing that predictor. A cutoff of 0.8 (indicated by the dashed line) was set to distinguish between important and nonessential predictors.

biomass: +31.83%, n = 19; 350−600 °C: +28.65%, n = 25; both $p < 0.002$), with fertilizers, and at medium biochar application rate (fertilizer: +49.99%, n = 4; 1−2.5% rate: +28.64%, n = 17; both $p < 0.01$) (Figures 3 and S2). Significant moderators of dehydrogenase activity were biochar feedstock, pyrolysis temperature, soil CEC, application rate, and experiment duration. Greater dehydrogenase activity increases occurred with agricultural biomass or lignocellulose feedstock (agricultural biomass: +86.90%, n = 69; lignocellulose: +84.00%, n = 23; manure: +67.03%, n = 36; all $p < 0.02$), low pyrolysis temperature (≤350 °C: +101.14%, n = 42; 350−600 °C: +74.63%, n = 86; both $p < 0.005$), high soil CEC (<9 cmol/kg: +28.96%, n = 40; >12 cmol/kg: +78.00%, n = 6; both $p < 0.001$), medium biochar application rate (<1% rate: +88.71%, n = 52; 1−2.5% rate: +74.69%, n = 76; both $p < 0.005$), and longer experiment duration (<15 days: −9.17%, n = 32, $p < 0.001$; 20−50 days: +93.77%, n = 96, $p = 0.609$) (Figures 3 and S2). Another meta-analysis also found low pyrolysis temperature resulting in significantly higher dehydrogenase activity, although it did not consider the fixed effects of moderators and had small observation sizes than this study.[11] For cumulative CO_2, the pyrolysis temperature was the only significant moderator. Low pyrolysis temperature resulted in greater increases in cumulative CO_2 (≤350 °C: +56.03%, n = 12, $p < 0.004$; 350−600 °C: +7.64%, n = 26, $p = 0.602$) (Figure 3). This trend is consistent with stronger metabolic responses of soil microbes, exemplified by the activity of the three C-cycling enzymes, to low-temperature biochar.

For N-cycling genes (*amoA* of AOB, *nirS*, *nosZ*), enzyme (urease), and processes (potential nitrification rate, cumulative N_2O), biochar feedstock, pyrolysis temperature, soil C/N ratio, fertilizer addition, application rate, experiment duration, and field or laboratory were significant moderators ($p < 0.05$ in omnibus test) (Table S6). No significant moderator was identified for AOA or *narG* encoding nitrate reductase ($NO_3^- \rightarrow NO_2^-$) (Table S6). For ammonium-oxidizing bacteria (AOB), fertilization was the only significant moderator. Together with fertilization, biochar increased AOB by nearly 3 times (no fertilizer: −7.75%, n = 48, $p = 0.892$; fertilizer: +277.45%, n = 13, $p < 0.05$) (Figure 3). Significant moderators for *nirS* included feedstock and experiment duration. Wood biochar and shorter duration resulted in larger *nirS* increases (wood: +13.54%, n = 20, $p < 0.004$; <15 days: +33.07%, n = 4, $p < 0.001$) (Figure S2). Significant moderators of *nosZ* were biochar feedstock and fertilization. Biochar generated from agricultural biomass increased *nosZ*, while wood biochar reduced *nosZ* abundance (agricultural biomass: +15.63%, n = 16, $p = 0.016$; wood: −2.97%, n = 20, $p = 0.673$) (Figure 3). Fertilization revoked biochar's positive effect on *nosZ* (no fertilizer: +15.00%, n = 23, $p = 0.013$; fertilizer: −6.46%, n = 13, $p = 0.407$). Significant moderators of urease activity included biochar feedstock, pyrolysis temperature, soil zone, biochar application rate, fertilization, and field or laboratory; however, only feedstock reduced total nonsampling variance. Urease activity had higher increases under medium application rate (<1% rate: +20.09%, n = 14, $p = 0.196$; 1−2.5% rate: +43.04%, n = 58, $p < 0.01$) and in the laboratory (laboratory: +42.32%, n = 60, $p = 0.025$; field: +31.46%, n = 14, $p = 0.301$) (Figure 3), and by biochar made from lignocellulose and at low pyrolysis temperature in the rhizosphere and without fertilization (Figure S2; also see the Supporting Information for results from <10 observations). For potential nitrification rate, experiment duration was the only significant moderator. Longer experiments increased the potential nitrification rate nearly 2 times more than shorter experiments (<15 days: +28.26%, n = 15; 15−20 days: +52.09%, n = 18; both $p <$ 0.001) (Figure 3). For cumulative N_2O emissions, significant moderators included feedstock, soil C/N, and fertilization (results from <10 observations in the Supporting Information).

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All feedstocks but wood reduced cumulative N_2O emissions (lignocellulose: −31.05%, n = 26, p = 0.014; wood: +24.01%, n = 36, p = 0.089) (Figure S2; also see the Supporting Information for other feedstocks with <10 observations), consistent with aforementioned increase in *nirS* and decrease in *nosZ* due to wood biochar. Reduced cumulative N_2O emissions occurred under a range of soil C/N (Figure S2). Fertilization curtailed biochar-induced inhibition of cumulative N_2O emissions (no fertilizer: −27.43%, n = 41, p = 0.293; fertilizer: 21.65%, n = 30, p = 0.343) (Figure 3), consistent with the aforementioned decrease in *nosZ* genes attributed to fertilization.

For P-cycling enzymes, biochar feedstock, pyrolysis temperature, and soil pH were significant moderators (p < 0.05 in omnibus test) (Table S6). Acid phosphatase activity was significantly increased by biochar made at low pyrolysis temperature (≤350 °C: +16.16%, n = 16, p < 0.045; 350−600 °C: −3.24%, n = 70, p = 0.610) (Figure 3). Alkaline phosphatase activity was enhanced by biochar made from agricultural biomass more than that by other feedstocks (agricultural biomass: +84.92%, n = 4, p < 0.001), and only in neutral to alkaline soils (pH < 5: −6.36%, n = 24, p = 0.694; pH 5−8: +27.29%, n = 14, p < 0.017) (Figure S2).

3.4. Microbial Response Predictors and Precision Biochar Soil Amendment. Feedstock, pyrolysis temperature, soil pH, fertilization, biochar application rate, and experiment duration were the most important predictors of the 24 microbial responses (Figure 4; Table S7) and were most frequently included in the final models (Table S8). Feedstock was an important predictor of bacterial PLFA, β-glucosidase, and cumulative N_2O emissions. Pyrolysis temperature was an important predictor of dehydrogenase, cumulative CO_2 emissions, and acid phosphatase activity. Soil pH was an important predictor for fungal PLFA, Chao1 diversity, and acid phosphatase activity. Biochar application rate was an important predictor for MBC, urease activity, and potential nitrification rate. Fertilization was an important predictor of AOB abundance and cumulative N_2O emissions. Experiment duration was an important predictor for potential nitrification rate and acid phosphatase activity.

By controlling important predictors of microbial responses, it is possible to implement precision biochar amendment to reach a specific soil amendment goal. When pursuing multiple goals, multicriteria decision analysis could be conducted to prioritize soil management outcomes. First, biochar production conditions can be tuned to elicit the desired microbial responses. Feedstock and pyrolysis temperature are major controls of biochar physicochemical properties such as porosity, specific surface area, crystallinity, fixed carbon, volatile and ash content, elemental composition, surface functional group, and recalcitrance.[59−62] Reduction of N_2O emissions can be better achieved with biochar made from lignocellulose or agricultural biomass but not wood biochar. Biochar made at lower pyrolysis temperatures is preferred for dehydrogenase and acid phosphatase activity that can increase soil CEC and thus nutrient retention; however, CO_2 reduction requires biochar made at a higher temperature. Switching biochar generated at different temperatures during the growth season may allow both goals to be achieved. Second, biochar amendment plan could be optimized to invoke the best outcomes. Higher application rate could enhance urease and nitrification rate more but may reduce acid phosphatase activity. Optimal amendment rates can be determined based on the needs of different crops. Moreover, our model showed that N_2O inhibition was stronger in shorter durations, suggesting that even with the optimal amendment plan, repeated biochar amendment may be needed for the best N_2O mitigation outcome.

3.5. Knowledge Gaps and Future Directions toward Biochar-Based Sustainable Soil Management. We identified several critical knowledge gaps hindering the realization of biochar-based sustainable soil management. First, a mechanistic understanding of biochar-induced soil microbiome alterations is still largely lacking. Multiomics holds the promise to shed light on genome-to-phenome changes in the soil microbial community.[10,63] The rhizosphere microbiome at the interface between plant roots and soil deserves particular attention as it links below-ground processes to above-ground plant development and health. However, our systematic literature review identified only three studies that focused on rhizosphere microbiome responses. Multiomics may also offer an in-depth understanding of N_2O inhibition by biochar, where multiple mechanisms have been proposed, including reduced denitrification due to aeration, adsorption of reactive N intermediates, shifted denitrification stoichiometry due to pH increase, and more complete denitrification by denitrifiers owing to available labile C or biochar-facilitated electron transfer.[22,64−66] Biochar can influence multiple pathways in microbially mediated N-cycling, which can eventually shift the $N_2O/(N_2 + N_2O)$ ratio.[24,64] Expression and activity of several reductases (NIR, NOR, and NOS) require particular attention. Second, biochar varies significantly in its physicochemical properties. The interpretation of biochar-induced soil microbiome changes requires considering the biochar properties. Specifically, biochar's redox activity is important to many microbially mediated transformations in soil, but it is not widely reported. Lignocellulose is an essential source of redox moieties, such as electron-donating phenolic moieties and electron-accepting quinones. Pyrolysis temperature influences the surface density of these moieties and the ordering of carbon structures, thus affecting charging and discharging of surface functional groups and direct electron transfer, which are the two electron flow pathways seen in biochar.[67,68] Future efforts should address, with tools such as machine learning, how to leverage biochar redox characteristics to evoke desired microbial responses such as N_2O mitigation. Overall, this study contributes to a deeper understanding of microbial responses to soil biochar amendment and highlights the promise of purpose-driven biochar fabrication and amendment in sustainable agriculture.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c04201.

Increasing number of biochar studies over the past decades, keyword combinations used in the systematic literature review, all primary studies used in this meta-analysis, complete list of response variables extracted from all primary studies, complete list of predictor variables used in this study, best model selection procedure, publication bias and missing data, full results of three-level random-effects models, additional results and dicussion about microbial responses with smaller numbers of observations, and funnel plots for global

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effect sizes of individual soil microbial responses before model selection and in their final models (PDF)

Raw data extracted from the 61 primary studies, full summary of statistics of the three-level mixed-effects models containing one single categorical moderator, importance of individual predictors, full summary of statistics of the final three-level mixed-effects models, assessment of publication bias in the three-level mixed-effects models containing one categorical moderator, and assessment of publication bias in the final best three-level mixed-effects models containing multiple moderators (XLSX)

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Notes

The authors declare no competing financial interest.

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